The 3rd APEC Workshop on Sustainable Agricultural Development
16th to 22nd November 2003, Chinese Taipei

Non-pesticide Methods for Controlling Plant Diseases and Insect Pests in Chinese Taipei

Tze-Chung Huang

Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan, *Chinese Taipei*

Non-pesticide Methods for Controlling Plant Diseases and Insect Pests in Chinese Taipei

Tze-Chung Huang

Bureau of Animal and Plant Health Inspection and Quarantine,
Council of Agriculture, *Chinese Taipei*

Abstract

Located in subtropical area, Taiwan has warm and humid climate that is suitable for the occurrence of plant diseases and insect pests. Farmers in Taiwan were used to controlling diseases and insect pests with pesticides that might result in problems of chemical residue, pesticide resistance and environmental pollution. In order to minimize the negative effects caused by the use of pesticides, plant protection institutions have started intensive researches and extensions of non-pesticide methods since early 1970's. The top authority of plant protection, Council of Agriculture (COA), has set the development and extension of non-pesticide methods as a national strategic plan since 1984. With the efforts over the past few years, we have promoted awareness of non-pesticide control, and brought many techniques into extensive application resulting in satisfactory outcomes.

Previously, we experienced considerable success in adopting non-pesticide measures to control insect pests. Among the most well known cases are introducing exotic parasitic wasps to control coconut leaf bud beetle and citrus plant lice, releasing indigenous parasitic wasps to control Asian corn borer. In recent years, some exotic predatory mites have been introduced into Taiwan and tested for their effectiveness of controlling spider mites, and exotic parasitic wasps were introduced to be tested for controlling silver leaf white fly that invaded Taiwan in 1989. Besides, indigenous natural enemies including parasitic wasps, green lacewing, and flower bug have been tested for their effectiveness of controlling different insect pests and some of them were considerably effective. Techniques for mass rearing of the promising natural enemies have been invented or improved that will make the extensive application of the biocontrol agents possible. Other non-pesticide methods that have been carried out in Taiwan include using sex pheromones to control cotton worm, beet army worm, diamond-back moth, sweet potato weevil and smaller tea tortrix, use of methyl eugenol and cuelure 〔4-(p-acetoxyphenyl-2-butanone)〕 mixed with insecticides to annihilate male oriental fruit fly and melon-fly, respectively, hanging yellow sticky cards to catch small insect pests such as white flies, thrips, leafhoppers and aphids. With regard to plant diseases, although its initiation was later, we have gained remarkable achievements. For example, controlling soil-borne diseases of high-value crops by steam sterilization, producing

pathogen-free propagating materials of banana, citrus, asparagus beans, etc. to prevent systemic diseases, especially that are caused by viruses, control of plant diseases with antagonistic microorganisms such as *Trichoderma* spp., *Bacillus subtilis* and *Streptomyces saraceticus*, control of plant parasitic nematodes with the amendment of LT-M mixture into soil, control of several fungal and bacterial diseases with the amendment of SH mixture and an organic compound, FBN-5A, mixed with BVB NO. 4 medium. Moreover, the use of resistant rootstocks for the control of soil-borne diseases also resulted in a success. By using loofah as rootstock, *Fusarium* wilt of bitter gourd is effectively controlled, and by using eggplant as rootstock, bacterial wilt of tomato is satisfactorily prevented. Genetic engineering has been applied to develop transgenic plants resistant to viral diseases in Taiwan. Field trials showed that 3 coat-protein transgenic papaya lines provided a broad-spectrum resistance to different *Papaya ringspot virus* strains originated from Hawaii, Thailand, and Mexico. Genetically-engineered mild strains of viruses were generated to control virus diseases by cross protection. Several projects supported by National Science Council or Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ), Council of Agriculture, aiming at developing transgenic plants resistant to different kinds of diseases are in progress.

Keywords: Non-Pesticide control, natural enemy, sex pheromone, steam sterilization, antagonistic microorganisms, transgenic papaya, genetically-engineered mild strain.

Introduction

Chemical pesticides have long been regarded as the most convenient and effective materials for the control of plant diseases and pests. Although the use of pesticides is unavoidable, high amount of consumption not only wasted money but also caused a lot of problems including chemical residue in foods, environmental pollution and adverse effects on ecosystem. Therefore, developing and applying non-pesticide methods as alternative to chemical control of plant diseases and pests has become a global trend. Located in subtropical area, Taiwan has warm and humid climate that is suitable for the occurrence of plant diseases and insect pests. Farmers in Taiwan are used to controlling diseases and pests with pesticides. In an era of increasing consciousness for food safety and environmental protection, the top authorities of plant protection, Council of Agriculture (COA), has set the development and extension of non-pesticide methods as a national mission in terms of plant protection since 1984. With the efforts through years, we has promoted awareness of non-pesticide control, and brought many techniques into extensive application resulting in satisfactory outcomes. This paper states the development and extension of non-pesticide methods for controlling plant diseases and pests in Taiwan.

Non-Pesticide Control of Insect Pests

Use of natural enemies

In Taiwan, natural enemies of plant insect pests have been used for biological control since early 20th century. Starting in 1947, an indigenous parasitic wasp, *Trichogramma chilonis* (Ishii) (Hymenoptera, Trichogrammatidae), had been studied for its effectiveness of controlling gray borer, stalk borer and shoot borer of sugarcane and has been extensively released since 1972 to sugarcane fields resulting in successful control (COA, 1996). However, extensive studies on biological control had not been common until early 1970's due to the prevalence of chemical control. Natural enemies that interest Taiwanese researchers, especially those who work at Agricultural Research Institute (TARI), include exotic and indigenous ones in terms of their origin. Many kinds of exotic natural enemies have been introduced into Taiwan and tested for their effectiveness of controlling domestic insect pests. Some of them performed well as control agents, whereas some of them failed to establish (Lo, 2002). The information concerning the introduction of exotic natural enemies is summarized in Table 1. In recent years, the natural enemies that have commonly been studied and applied in Taiwan included parasitic wasps, predatory mites, green lacewing, and flower bugs. Some of them have been extensively applied with satisfactory results and some of them are still limited to small-scale field trials.

1.Parasitic wasps

Citrus plant lice, *Diaphorina citri* (Kuwayana) (Homoptera: Psyllidae), is a vector of citrus Likubin in Taiwan. Data showed that it could be effectively controlled by nymphal parasitoid, a parasitic wasp, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), in Reunion Islands. The parasitic wasp was introduced into Taiwan to control citrus plant lices. A total of 64 adult parasitoids in 4 shipments were obtained from 1983 to 1986 and subsequently a total of 21,164 adults of *T. radiata* were propagated and released to 39 citrus groves and common jasmine orange (*Murraya paniculata* Jack. var. paniculata) bushes in 12 counties during 1984-1988. Survey data showed that the wasp has successfully established in this island and kept citrus plant lice at low density (Chien *et al.*, 1989).

Coconut leaf bud beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) was a serious pest of coconut palm in Taiwan during early 1980's. Its larval and pupal parasitoid, a parasitic wasp, *Tetrastichus brontispae* (Ferr.) (Hymenoptera: Eulophidae), was introduced into Taiwan from Guam in November 1983. A total of about 143,082 adults were released by 106 times to Kaohsiung, Pingtung, Taitung, Hualien, Taitung, Chiayi, Yunlin and Taipei from January 1984 through June 1986. It was evident that the introduced parasitoid had already established in Taiwan and performed as an excellent biological control agent of the coconut leaf beetle. Survey data indicated that this

biological control project was a great success (Chen, 1992b).

An indigenous parasitic wasp, *Trichogramma ostrinae* (Pang & Chen) (Hymenoptera: Trichogrammatidae), has been used as a biological agent to control Asian corn borer , *Ostrinia furnacalis* (Guenée) (Lepidoptera: Pyralidae) , the most important insect pest of corn in Taiwan, since 1984. So far, it has been applied to a total of 200,000 hectares of corn resulting in a considerable success in terms of effect on direct control of the pest and significant reduction of the use of pesticides. The wasp was propagated to a large number with the eggs of rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae), as substitute hosts. An egg card machine was developed to improve the efficiency of producing cards stuck with eggs of the rice moth harbored by the wasp. Production of the egg cards with this machine is 10 times faster than that by laborers. It saves 2,626 hours and 4,700 US dollars in every lot of 3,000,000 egg cards (3.5 x 1.5 cm) that are used to release the parasitoid to 10,000 hectares of corn for controlling Asian corn borer (Chen, 1992a; COA, 1996). The improvement of technique for mass production will facilitate the application of the biocontrol method.

2. Green lacewing

Green lacewing, *Mallada basalis* (Walker) (Neuroptera: Chrysopidae), is an indigenous predatory natural enemy of several kinds of plant insect pests including spider mites, whiteflies, leafminers, thrips, and aphids. Successful control of those pests using the larvae of the green lacewing was achieved on citrus, muskmelon, papaya, jujube, and strawberry in screen houses and in the open fields as well. To improve the efficiency of mass rearing of the green lacewing, a prototypical machine with control of temperature, pressure and fluid level was developed to make microcapsulated artificial diets. The microcapsules about 396±48 μm in diameter and 10 μm in thickness were produced with 90% success rate. When *M. basalis* larvae were reared with the microcapsulated artificial diets modified from Hassan and Hagen's formula, 90% of individuals reached the adult stage in 20.7 days and the females deposited 348.5 eggs on average. The results were comparable with those reared with eggs of *Corcyra cephalonica* or *Aphis glycines* (Matsumura) (Hemiptera: Aphididae) (TARI, 1999). The improvement of rearing the predatory biocontrol agent will make its large-scale extension possible.

Another green lacewing, *Chrysopa boninensis* (Okamoto) (Neuroptera: Chrysopidae), is a polyphagous predator. Larvae are known to feed on a wide variety of small, soft-bodied insects such as aphids, scales insects, mealybugs, plant lices, spider mites, and on eggs and small larvae of a number of lepidopteran insects. *Chrysopa* was thus chosen as an agent for citrus red mite control. A preliminary experiment was conducted in a citrus grove. One thousand eggs of *Chrysopa* were released per tree. Twenty days later, the population density of citrus red mites in the released plot was much lower than in the control plot. The level of predation was sufficient to greatly reduce the mite population and keep it down to a very low level (TARI, 1999).

3. Flower bug

An indigenous predatory flower bug, *Orius strigicollis* (Poppius) (Hemiptera: Anthocoridae), had proved to be a good predator of small insects, such as thrips, and spider mites in Taiwan. Large number of *Orius* flower bug can be propagated under constant temperature and photoperiod conditions with bean [*Glycine max* (L.) Merill]sprouts as food and ovipositional substrate and flour moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) eggs as prey. Field-release tests were conducted on eggplant and adzuki bean grown in open fields in central and southern Taiwan in 1998 and 1999 to evaluate the effect of *O. strigicollis* on biological control. In the biocontrol plots, densities of thrips gradually declined after the release of the flower bugs. The number of thrips in the chemical control plots dropped immediately after insecticide applications, but increased rapidly afterwards. Significant differences between biocontrol plots and chemical control areas appeared 4-6 weeks after the first release. The control effect lasted for several weeks. It has proved, that the flower bug, when released at the proper timing, is a strong natural enemy for the control of thrips on certain crops in open fields (TARI, 1999).

4.Predatory mites

Predatory mites, *Amblyseius fallacis* (Garman) (Arachnida: Phytoseiidae) and *Phytoseiulus persimilis* (Athias-Henriot) (Arachnida: Phytoseiidae), have long been regarded as promising biocontrol agents of plant spider mites. They were introduced from the USA and trials to evaluate their usefulness were carried out in three commercial strawberry fields for controlling two-spotted spider mite, *Tetranychus urticae* (Koch) (Arachnida: Tetranychidae), in Taiwan over three seasons during 1986-1989. Cooperating growers controlled diseases, mites and insect pests by means of a weekly or biweekly spray schedule before the fruiting stage. When the strawberry plants began to blossom, all chemical applications were stopped. *A. fallacis* and *P. persimilis* were released at a rate of 10/plant, at the time when two-spotted spider mite reached 2 active individuals/leaflet or infested 30% of sampled leaves. Satisfactory control of *T. urticae* was achieved when enough predatory mites were released and /or selective acaricides were applied as auxiliary control agents (Ho and Lo, 1992). The establishment of an integrated pest management system based on the biological control of *T. urticae* by both two predatory mites is in progress.

Both of the abovementioned predatory mites also gave effective control of Kanzawa spider mite, *Tetranychus kanzawai* (Kishida) (Arachnida: Tetranychidae), the most common spider mite on papaya. *P. persimilis* was more effective than *A. fallacis* when 500 predators/plant were released each time for two or three times in the test fields. The best releasing position on plant was found to be the lower leaves. The results showed that spider mite could not be found in the biological control plots. However, 41.4 mites and 469.0 mites/leaf were found in chemical control plot and untreated plot, respectively (Lo *et al.*, 1990). *A. fallacies* was also tested for its effectiveness of controlling Kanzawa

spider mites (*T. kanzawai*) on mulberry. A total of 5,271 million *A. fallacis* were mass produced, and released to 179.33 ha. of mulberry orchards. Encouraging results were obtained at Miaoli, Natou, and Chiayi. Moreover, *A. fallacies* has been found to be well established in pear trees and gave effective control of both species of spider mite. During the winter, the predatory mite migrated down into the ground cover, where it lived on broadleaf weeds and continued to prey on the two-spotted spider mite (Ho and Chen, 1992; Ho and Lo, 1992)

Use of sex pheromones

In order to enhance the application of non-pesticide method in the field, our government has financed the researches and extension of using sex pheromones for controlling the insect pests on many crops since 1983. The kinds being promoted included sex pheromones of tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae); beet army worm, *Spodoptera exigua* Hübner (Lepidoptera: Amphipyrinae); sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae); carambola fruit borer, *Eucosma notanthes* (Meyrick) (Lepidoptera: Tortricidae); smaller tea tortrix (*Adoxophyes* sp.); rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae); tomato fruit worm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae); turnip moth, *Euxoa segetis* (Schiffermiller) (Lepidoptera: Noctuidae); Asian corn borer, *Ostrinia furnacalis* (Guenée) (Lepidoptera: Pyralidae) and cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). Different kinds of sex pheromones were applied to crucifer, shallot, peanut, soybean, flowers, sweet potato, corn, carambola and tea, respectively.

In practice, they were used mainly to monitor or mass trap the target insect pests and occasionally to disrupt mating. Among them, the sex pheromone of sweet potato weevil has been most extensively applied in the fields. A funnel-shaped bottle suitable for trapping sweet potato weevil was invented and patented. The traps containing the sex pheromone lure were set in the fields at the rate of 40/ha. and the density of the weevil was reduced by 55~65% (Hwang and Hung,1991). This method has been applied to a total of 12,000 ha. of sweet potato since 1991. Besides, The sex pheromones of carambola fruit borer, Z-8-dodecenyl acetate and Z-8-dodecenol, had also been intensively studied. When they were used for mass trapping (40-50 traps/ha. lasting for 5 months), the density of the fruit borer was reduced by 75~89% as compared to untreated orchards. Assessment of using sex pheromone for mating disruption of carambola fruit borer was studied in the cage and orchards of small acreage. Data displayed that when the ratio of pheromones were properly formulated and the amount used were sufficient (53.28 g/ha), the mating inhibition in the orchards reached 97.45%. The fruit damage was reduced by 71% as compared to conventional chemical control (Hung and Hwang, 1993). The information pertaining to sex pheromones and attractants of insect pests is summarized in Table 2.

Other non-pesticide methods

Male annihilation by using materials other than pheromones has also been extended in Taiwan. Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), has always been a serious problem on several kinds of fruits. Different approaches to controlling Oriental fruit fly had been compared and using sugarcane-fiber plate absorbed with methyl eugenol (95%) and Naled (5%) for male annihilation was found to be the most effective and convenient method (Liu, 1992). Subsidized by the government, this method has been applied to about 580,000 ha. of orchards and groves since 1998. Melon-fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae), has long been the key pest of cucurbits. Cuelure〔4-(p-acetoxyphenyl-2-butanone)〕mixed with insecticides has been recommended for male annihilation and commonly adopted by the farmers (Chen and Chang, 1993). Yellow sticky cards are commonly employed by farmers to trap small insect pests such as white flies, thrips, leafhoppers and aphids for monitoring and/or control purposes (Wang and Lin, 1992).

Pathogenic microbes including bacteria, viruses, and nematodes are regarded as promising biocontrol agents of insect pests. However, *Bacillus thuringiensis* Berliner has been the only microbe commonly used in Taiwan so far. It was first officially recommended to control diamond-back moth and then Asian corn borer (Hsiao, 1984.) Further studies showed that when mixed with fried rice bran, its efficacy of controlling Asian corn borer was significantly improved (Wu and Tseng, 1997).

Non-Pesticide Control of Plant Diseases

Physical control

1. Mulching and screen housing

There are two physical methods that are commonly used in Taiwan to prevent weeds, diseases or insect pests. For planting papaya, seedlings free from *Papaya ringspot virus* (PRSV), the limiting factor of papaya production in Taiwan, are planted in screen houses that prevent aphids, the transmission vectors of PRSV, from entering. The soil is covered with plastic sheets that prevent the growing of weeds. By this way, papaya has been stably produced at about 138,600 tons per year since early 1990's (Wang and Wang, 1995). Mulching of soil with plastic sheets is also commonly adopted by the growers to cultivate many kinds of crops such as watermelon, muskmelon, cucumber, tomato, eggplant, pepper, strawberry, etc. By this way, weeds are mostly inhibited and the occurrence of insect pests and soil-borne diseases are also reduced to some extent. Besides, the simple facilities, tunnels constructed with transparent plastic sheets under which muskmelon is planted, are also commonly adopted by the growers. By this way, viral diseases, downy mildew and some insect pests are effectively controlled (Huang *et al.*, 1999)

2. Steam sterilization

Recently, plant disease control by steam sterilization was used in planting high-value horticultural crops in Taiwan. Soils and cultural composts contaminated with plant pathogens were sterilized by the injection of steam (60-80℃ for 30 min). Plant diseases, such as lily yellowing disease caused by *Fusarium oxysporum* Shlechtend: Fr. emend f. sp. *lilii* Imle, *Thanatephorus cucumeris* (Frank) Donk, bacterial wilt of lisianthus caused by *Ralstonia solanacearum* Yabuuchi *et al.*, southern blight of ornamentals caused by *Athelia rolfsii* (Curzi) Tu & Kimbrough, etc., were controlled significantly by steam sterilization. In addition to the disease control, the quality of ornamental products and weeds control were also improved. Some horticulture growers, such as those who plant lisianthus and potted flowers, by the assistance of plant pathologists, developed their own steam pipe systems to improve the efficiency of operation in fields and screen houses (Lee, 1997). Although this method is labor-costly and time-consuming and therefore did not appeal to some growers, the benefits of reducing both the use of pesticides and adverse impacts on the environment still make it promising in the future, especially in the ornamental industry.

Use of pathogen-free propagating materials

Since seeds and seedlings are the starting materials for the production of crops and might be the carriers of pathogens and pests, the use of healthy seeds and seedlings is one of the most important strategies for non-pesticide controlling methods in many countries. By using healthy seeds and seedlings, the crop productivity and quality can be increased and improved. In Taiwan, the system of healthy seeds and seedlings production was started from the propagation and extension programs for seeds of the staple crops such as rice and coarse grains such as corn, soybean, peanut, and etc. In 1959, the Seed Testing Laboratory (STL) was established for the inspection of fields for foundation, registered, and certified seeds production and laboratory testing of the quality including their purity, moisture content, germination rate and health. The acreage of fields, mainly paddy rice, inspected amounted to about 2,000 hectares annually over the years (Kao and Lee, 2001).

Subsequently, Other propagation and inspection systems of virus-free seedlings of other crops were taken effect. Among them, the systems for potato, strawberry and sweet potato were established in 1972, 1991 and 1998 respectively. In the systems, STL are engaged in all the inspections and testing, and TARI, Chiayi Agricultural Experiment Branch Institute, and Taiwan Seed Improvement and Propagation Station (TSIPS) are responsible for the supplies of foundation seeds of sweet potato, potato and strawberry respectively. The inspection systems of those three crops operated well and were successful in supplying high quality seeds and seedlings (Chang and Lee, 1991; Kao and Lee, 2001).

The production technique of virus-free seeds of asparagus bean had been

developed by TARI and worked in practice by TSIPS. The extension area of healthy seeds has amounted to 824 hectares since 1996 (Kao and Lee, 2001). With regard to passion fruit and bamboo, TARI and Tainan District Agricultural Improvement Station are responsible for, respectively, the inspection and maintenance of virus-free nuclear stock repositories founded to meet the needs of commercial nurseries. By estimate, 250,000 virus-free passion fruit seedlings have been released per year since 1989 (Chang, 1991), and 60,000 healthy bamboo seedlings free from *Cucumber mosaic virus* have been provided since 1992 (Cheng and Yeh, 2001). With regard to citrus, the system is carried out mainly by Chiayi Agricultural Experiment Institute with technical support from National Taiwan University. Pathogen-free budlings of citrus are provided by Chiayi Agricultural Experiment Institute to meet the needs of commercial nurseries. In recent years, over 20,000 pathogen-free budlings of citrus were produced annually (Su, 2001). In addition, Taiwan Banana Research Institute and Taiwan Sugar Company take charge of the propagation and indexing of healthy seedlings of banana and sugarcane in Taiwan. The amount of healthy banana seedlings, produced by tissue culturing, supplied was estimated to 39,000,000 during 1983-2002 (Hwang, 2001; Kao and Lee, 2001).

Furthermore, to comply with the "Guidelines for Inspection of Designated Plant Diseases and Pests on Anthurium Seedlings", a mandatory inspection to ensure that the propagating materials are free from quarantine nematodes and bacterial blight pathogen, *Xanthomonas campestris pv. Dieffenbachiae* Vauterin *et al.* has been implemented since September, 1990 and over 900,000 healthy seedlings have been certified so far (Chang and Lee, 1991; Kao and Lee, 2001). Besides, our government is in the process of establishing of voluntary seed and seedling certification systems to encourage the production of high quality and healthy seeds. The certification system for *Oncidium* orchid seedling has taken effect since 2002 and asparagus bean, green bamboo and *Phalaenopsis* orchid will be the following ones to be promulgated this year. In the future, many more crops will be included in the voluntary seed and seedling certification system when the techniques are available. It is expected that the implementation of the voluntary seed and seedling certification system will significantly enhance the quality of seeds and seedlings of related crops and subsequently promote the competitiveness of Taiwan's agriculture (Chang and Lee, 1991; Kao and Lee, 2001).

The follows are the detailed descriptions regarding some of the prominent cases that used pathogen-free propagating materials to control major diseases in Taiwan.

1. Application of tissue culture technology for controlling *Fusarium* wilt of banana

Fusarium wilt of Cavendish banana caused by *Fusarium oxysporum* Schlechtend: Fr. emend f. sp. *cubense* (E. F. smith) Snyder & Hansen race 4 has been the major constraint for banana production in Taiwan. Previously, the disease had destroyed the banana industries of many countries in Central

America despite many attempts to control the disease. Continued banana production in Taiwan in recent years to supply both local and the Japanese market was attributed to the successful development of tissue culture technology for controlling *Fusarium* wilt. There have been two major achievements based on tissue culture technology leading to the success of controlling *Fusarium* wilt. Firstly, the technology was employed for mass propagation of disease-free plantlets for commercial planting. During 1983-2002, the tissue culture program had produced a total of 39 million plantlets for planting over 19,493 ha. and effectively contained the spread of wilt pathogen via infected suckers traditionally used by farmers. Secondly, because propagation of banana by tissue culture has shown the potential of producing substantial genetic variability, a mass screening program to select tissue culture plants resistant to *Fusarium* wilt was taken. Two resistant varieties, Tai-Chiao No.1 and Tai-Chiao No.3, developed by this breeding program were released for controlling *Fusarium* wilt in 1992 and 2001, respectively. Another new, higher yielding, resistant variety, Formosana, selected most recently was released in 2002. It is estimated that planting Formosana to replace Giant Cavendish would have the potential to increase yield by over 50%, and thus boost banana production considerably (Hwang, 2001; Hwang and Su, 1998).

2. Production and extension on *Bamboo mosaic virus*-free clones/plants of green bamboo

Because green bamboo shoot is rich in nutrition and delicious, it has been considered an important vegetable especially during the summer. *Bamboo mosaic virus* is the most destructive disease affecting the cultivation of bamboo shoots. The percentage of infection on green bamboo based on visible symptoms was 80-98.8 in the major growing areas of green bamboo. Previous studies revealed that the disease was transmitted mainly by cultural practices via farming tools. The control for the disease by planting the virus-free clones was effective. Since 1989, eye-inspection and/or the ELISA methods were used for the indexing of the disease, and green bamboo virus-free mother plant fields were set up at Tainan DAIS Hsinhua Branch Station in 1990. A virus-free clones nursery was set up at Paiho, and has been indexed periodically once a month and the new clones were distributed to the bamboo farmers at their request. This nursery has produced 60,000 virus-free clones for farmers so far (Cheng and Yeh, 2001; Kao and Lee, 2001). The establishment of the pathogen-free seedling system has significantly improved the quality and ensured the stable production of green bamboo shoots. This system will also facilitate expanding the international markets of green bamboo shoots.

3. Production and application of virus-free seeds to control virus diseases of asparagus beans

Asparagus bean, *Vigna sesquipedalis* (L.) Fruwirth, is one of the important vegetables in Taiwan in hot and wet summer seasons. Among various kinds of diseases attacking asparagus bean, viral diseases are identified as major limiting factors for its production. *Cucumber mosaic virus* (CMV) and *Blackeye cowpea*

mosaic virus (BLCMV) were previously found as the two major viruses attacking asparagus bean in fields. Both viruses were found seed-borne and the infected seedlings could serve as primary inoculum for the consequent early epidemic as resulted form mechanical and aphid transmission. Investigation was directed to evaluate the effects of planting virus- free asparagus bean seeds in controlling the viral disease in fields. An asparagus bean cultivar "San-Tse-Chin-Pi" was selected for production of virus-free seeds in 1990. By rouging virus-infected plant after detection by ELISA using antisera against BLCMV and CMV, plants were cultivated in insect proof screen houses until seeds were harvested. The seeds were then served as foundation virus-free stock. A large-scale virus-free seed propagation system was subsequently developed in order to satisfy sufficiently the demand from the growers in Taiwan. In 1991-1992 we conducted four field trials to test the feasibility of using virus-free seeds to control viral diseases of asparagus bean. The result indicated that the application not only significantly delayed the virus epidemic but also increased the yield and quality of bean pods and eventually the profit earned by the growers. In the field experiments, as low as 30-72% of final virus incidences was recorded in the plots sowed with virus-free seeds while in the same time 100% of incidences were detected in the control plots planted with commercial seed lots (Chang, C. A. 2001). Since 1993, planting of virus-free seeds has been routinely taken as one of the key measures in the integrated pest control program of asparagus bean in various production areas. Because of the consistent and significant control effect the virus-free asparagus bean seeds, produced in this program, have become more and more popular in Taiwan with time. About 1,086 kg of virus-free asparagus bean seeds were produced and released to four different major asparagus bean producing counties in 2001 (Kao and Lee, 2001).

Use of antagonistic microorganisms

1.Control of plant diseases with antagonistic bacteria

Bacillus subtilis Cohn, a gram-positive endospore-forming bacterium ubiquitous in soil and many plant surfaces, has long been used as a beneficial microbial agent for the control of plant diseases. The fungicidal application of *B. subtilis* was one of the primed focuses of biopesticide product development during the past decade and attempt of commercialization development was increasing. In Taiwan, A *B. subtilis* isolate was recently commercialized by Kuan-Hwa Chemical Co.. The product has been registered for the control of powdery mildew of pea, whereas its potential market remains to be evaluated. In order to explore the usefulness of native bacilli resources, *Bacillus* spp. with broad spectrum of antagonistic activity against various phytopathogenic fungi and bacteria were selectively isolated from cultural substrate and soil samples collected from field. Among them, isolate BS1 of *B. subtilis* was found outstanding because of the superior antagonistic activity against *Rhizoctonia solani* Kűhn, *A. rolfsii* and *Xanthomonas campestris pv. mangiferaeindicae* Robbs *et al.*. Its mass production techniques were developed. As for the practical

uses, more than 30 crop species have been tested in greenhouse and/or field trials. Effective control of soil-borne and foliar diseases has been demonstrated, and significant growth promoting effect was generally observed. The efficacy of disease control apparently had certain connection to the production of iturin-like antibiotic. By spraying application of the liquid formulation at 500-1000 x dilution, effective control of foliar infection of tobacco by *Cercospora* sp. (tobacco white spot disease), and leaf and fruit infection of mango by *X. campestris pv. mangiferaeindicae* (bacterial spot of mango) were demonstrated by field trials. In cooperation with private enterprises, the commercialization of BS1 isolate is in progress (Tzeng et al., 2001). *Streptomyces* spp., being gram positive also, are found ubiquitous among various terrestrial and aquatic natural habitats. The members of Streptomyces mostly are known to be saprophytic, aerobic, exist commonly in soil, grow well in slightly alkaline environment, and adapt well in high temperature and drought conditions. As many *Streptomyces* spp. are known to produce important antibiotic secondary metabolites and hydrolytic enzymes, the wide and extensive industrial application has been well recognized. In Taiwan, a *Streptomyces saraceticus* Berger *et al.* isolate, SS31, was selected and was shown to be effective in knocking down root knot nematode population in soil primarily because of its chitinase producing characteristics. In a preliminary screening test, it was noted that the test isolate was strongly antagonistic against fairly wide spectrum soil-borne fungal pathogens—especially members of Oomycetes. When solid formulation of SS31 was added to a soil amendment consisting mainly spent forest mushroom compost, fish meal, crab shell powder, and rapeseed pomace, and amended into the soil, the mixture effectively controlled *Fusarium* wilt of lettuce, and *Fusarium* wilt of banana. By spraying application of the liquid formulation at 1000x dilution, effective control of *Phytophthora* diseases of papaya and citrus has been demonstrated. It was also found effective for the control of downy mildews on cucurbit, rose, grapevine, etc. In addition, the formulation was found useful as soil drenching agent for the control of soil-borne diseases. The drenching treatment of the liquid formulation at 100 to 300x in dilution significantly reduced damping-off of cucumber seedlings caused by *Pythium aphanidermatum* (Edson) Fitz. In cooperation with private enterprises, the commercialization of SS31 isolate is in progress (Tzeng *et al.*, 2001).

2. Control of plant diseases with antagonistic fungi

In Taiwan, *Trichoderma viride* Persoon:Fries and *Trichoderma koningii* Oudemans had been reported to be pathogenic to mushroom in 1967 and water chestnut in 1955, respectively. However, most of species/ strains of *Trichoderma* were found to be the effective microorganisms for suppression of the plant pathogenic fungi after 1980. During 1980-1990, antagonistic *Trichoderma* spp. were studied and the promising ones were selected, formulated and added into soils and found to be effective on the control of several plant diseases including *Rhizoctonia* diseases, *Sclerotinia* disease, *Pythium* damping-off and *Phytophthora* blight. Among them, *T. viride* T20, *T. knoningii* T12, *T. knoningii*

T12-R33-D25, and *Trichoderma harzianum* Rifai TVCN2 were tested in large-scale trials. The results showed that they could inhibit *Rhizoctonia* disease of chrysanthemum and adzuki-bean (Liu, 1991). Recently, it was found that *T. virens* strain R42 and *T. harzianum* T22, when added to the soils, could induce cucumber resistance to *Cucumber green mottle mosaic virus* and suppress the sudden death of melon caused by *Monosporascus* spp. Both of the biocontrol agents also promoted the seedling and root growth of the crops in greenhouse and field trials. In addition, two isolates, *T. harzianum* T2 and *T. virens* T3 that had been formulated as granules, were found to be effective on the control of dollar spot of turf grasses caused by *Sclerotinia homoeocarpa* Bennett, when the dose applied had reached 10^6 cfu/g soil dry weight (Lo, C. T. 2001). The techniques for the mass production of the antagonistic fungi have been exploited.

Use of soil amendments

Eight formulated soil amendments, S-H mixture, SF-21 mixture, L-T mixture, AR-3 mixture, CH-1 mixture, GS mixture, FBN-5A and CF-5 liquid, have been developed, mostly by plant pathologists at National Chung Hsing University, and used for the control of soil-borne plant pathogens in Taiwan. The ingredients of these compounds are mainly fertilizers and organic wastes. Among them, S-H mixture and L-T mixture have been commonly used and FBN-5A is ready for commercialization.

1. Control of Fungal Diseases with S-H mixture

S-H mixture, developed in 1983, has been commercialized in Taiwan for the control of soil-borne diseases. Amendment of soil with S-H mixture, significantly reduced the incidence of watermelon wilt caused by *Fusarium oxysporum* Schlechtend: Fr. emend Snyder & Hansen f. sp. *niveum* (E. F. Smith) Snyder & Hansen, radish yellows caused by *Fusarium oxysporum* Schlechtend: Fr. emend f. sp. *raphani* Kendric & Snyder, pea wilt caused by *Fusarium oxysporum* Schlechtend: Fr. emend f. sp. *pisi* (van Hall) Snyder & Hansen, *Fusarium* wilt of banana caused by *F. oxysporum* f. sp. *cubense*, clubroot of crucifers caused by *Plasmodiophora brassicae* Woronin, cucumber damping-off caused by *P. aphanidermatum*, southern blight of pepper caused by *A. rolfsii*, *Rhizoctonia* blight of bean caused by *R. solani* and bacterial wilt of tomato caused by *R. solanacearum*. The effective rate of application for The effective rate of application for S-H mixture varied with diseases and crops, ranging from 0.5 to 2% (w/w) of soil. In more than 5,000 ha. of field trials in a variety of vegetable crops, S-H mixture not only reduced disease incidence but also improved root health, plant growth and plant stands (Sun and Huang, 1983; Sun and Huang, 1985).

2. Control of *Rhizoctonia* damping-off with FBN-5A

Formulated in Taiwan, FBN-5A is a mixture of spent forest mushroom compost, fish meal, bone meal, blood wastes, lime and allyl alcohol which is

effective in controlling *R. solani* AG-4, *Sclerotinia sclerotiorum* (Lib.) de Bary and reduce the incidence of *F. oxysporum* f. sp. *raphani* and *Fusarium oxysporum* Schlechtend: Fr. emend f. sp. *lactucum*. FBN-5A at 1% also inhibits the seed germination of weeds such as purslane, black nightshade, etc. The control effect of FBN-5A is due to the ammonia released from chitin and blood meal that poison and weaken the mycelia. The microorganisms and some inhibitors exiting in the mixture also reduce the colonization on cabbage seedlings by *R. solani* AG-4. When FBN-5A was mixed with the commercial BVB No. 4 medium, composed of 30 % black peat, 10% sand, and 70% white Germany peat, at the rate of 0.1%, the treatment was very effective in controlling damping-off of cabbage caused by *R. solani* AG-4. It not only reduced the disease incidence, but also enhanced the growth of cabbage. The results were confirmed in a large-scale experiment in a commercial automatic environment-controlled greenhouse (Huang and Huang, 2000). This study suggests that the FBN-5A compound offers potential for commercial use as an organic amendment for the management of *Rhizoctonia* damping-off vegetable crops. In 2000, a patent was issued on the formula of FBN-5A, and is about to licensed to industry for further commercialization.

3. Control of plant parasitic nematodes with LT-M

In Taiwan, parasitic nematodes have been big problems on different kinds of crops. A mixture, Lively Tiller Mixture (LT-M) consisting of 40% crab shell powder, 40% castor pomace, 5% soybean powder, 10% marine algae powder and 5% molasses was formulated. When amended into soil, the mixture significantly inhibited *Meloidogyne incognita* Chitwood, *Tylenchlus semipenetrans* Cobb and *Pratylenchus coffeae* Filipjev & Schuurmans Stekhoven. It was able to increase the population of nematode-trapping fungi such as *Streptomyces saraceticus*. Among the ingredients, castor pomace and crab shell powder were toxic to the nematodes. Hatching of egg masses or dispersed eggs was inhibited by castor pomace. The amendment of marine algae powder made the roots of host plants repellent to juveniles of *M. incognita*, *T. semipenetrans*, *P. coffea* and *Paratylenchus curvitatus* van der Linde (Tsay, 1997; Tsay, 1998).

When applied at the rate of 2,000 kg/ha. to grape vines, LT-M significantly decreased the root-knot indices and disease indices by 47.5, 67.8 and 42.8, 69.5% at summer and winter harvest, respectively, as compared to untreated control. Besides, LT-M also increased weight of grape by 57, 76 grams per cluster and increased sugar content by 3, 5 Brix°at the two harvest seasons, respectively. Antinematodal effect was enhanced when LT-M was applied for 2 successive years. LT-M in soil of citrus groves also reduced the population of *T. semipenetrans* and *P. coffeae* by 94.1 and 63.6% respectively, as compared to the control. Application of LT-M again significantly controlled root-knot disease of African daisy (Tsay, 1997; Tsay, 1998).

Use of resistant rootstock

1. Resistance of bitter gourd-loofah grafts to *Fusarium oxysporum* f. sp. *momordicae*

The *Fusarium* wilt of bitter gourd caused by *Fusarium oxysporum* Schlechtend: Fr. emend f. sp. *momordicae* Sun & Huang is hard to control in the field (Lin *et al.*, 1996). The technique of cleft grafting to a loofah rootstock was developed and adopted in Taiwan.

F. oxysporum f. sp. *momordicae* can only attack bitter gourd and summer squash, but not other cucurbits that have the potential to be used as resistant rootstock for bitter gourd (Lin *et al.*, 1996). The grafting experiments showed that among the various cucurbit plants tested, only pumpkin, bottle gourd and loofah could be used as rootstock for successful grafting with bitter gourd. Loofah was found not only the best candidate as rootstock for having a highest survival rate in plastic houses and displaying vigorous growth in fields but also showed high resistance to the wilt pathogen even when inoculated by artificial root-pruning method. For three years of field cultivation, the combination of bitter gourd scion (variety of Known You New No.3) cleft grafted to loofah rootstock (a local line of L39) was the best one to have high bitter gourd yields which reached 61-108 mt./ha.. In contrast, the non-grafted bitter gourd or grafted seedlings by whip root grafting only gave a yield from none to 24 mt./ha. (Lin *et al.*, 1998). This method has been commonly adopted by the growers in Taiwan.

2. Control of soil-borne diseases of tomato by grafting on resistant eggplant rootstock

The planting area of tomatoes in Taiwan measures about 4,000 hectares with the cultivated season during autumn and winter. In recent years, in order to meet the need of the market, cultivation of tomato within protective facilities during summer time has become more and more prevalent in southern Taiwan. Under high temperature and humid conditions, soil-borne diseases such as bacterial wilt caused by *R. solanacearum*, root-knot nematode caused by *M. incognita* and *Fusarium* wilt caused by *Fusarium oxysporum* Schlechtend: Fr. emend f. sp. *lycopersici* (Sacc.) Snyder & Hansen took place and caused severe losses.

To eliminate the greatest limiting factor in the production of tomatoes, Tainan Agricultural Improvement Station and Asian Vegetable Research and Development Center (AVRDC) have cooperated to develop practical control measures since 1998, and have obtained some encouraging results. Currently, TA 6 is the main cultivated variety of tomatoes which is resistant to high temperature and *Tomato rugose yellowing virus* (TRYV), but very susceptible to root-knot nematode. With regard to the widespread and persistent characteristics of soil-borne diseases, the researchers focused on screening varieties of eggplants for compatible resistant rootstocks. They found that grafting of tomatoes on eggplant rootstocks, EG190, EG203 or EG219, could provide

resistance to nematodes, and increased the yield by 8% to 43.3%. The resistant eggplant rootstocks were grafted with Known-You 301 cultivar of tomato, which was susceptible to bacterial wilt, and bacterial wilt was significantly reduced as well (Chen et al., 2001).

Genetic engineering

In Taiwan, several measures have been adopted trying to control plant viral diseases. The measures include the application of integrated cultural practices to escape the transmission vectors, the utilization of artificially induced mild strains for cross protection (Wang et al., 1992; Wang et al., 1987) and the generation of transgenic plants resistant to specified viruses by genetic engineering. Among them, the studies on the control of *Papaya ringspot virus* (PRSV) by genetic engineering have reached the most remarkable achievement.

1.CP-transgenic papaya

PRSV is a major limiting factor for papaya (*Carica papaya* L.) production in tropical and subtropical areas throughout the world. Control measures that has been used to protect papaya plants from PRSV infection, including selection of planting time to avoid the peak of winged aphids, the use of silver mulch to prevent aphids from visiting seedlings, and conventional cross protection. None of these control methods provided a long period of effective protection against PRSV. The CP gene of a local mosaic type strain isolated from Taiwan, designed PRSV YK, has been sequenced, and transferred into papaya via *Agrobacterium*-mediated transformation by Dr. S. D. Yeh, at NCHU, and 45 putative transgenic lines were obtained. When the transgenic lines were challenged with PRSV YK by mechanical inoculation, they showed different levels of resistance ranging from delay of symptom development to complete immunity. After the evaluation of resistance under greenhouse conditions, four highly resistant transgenic papaya lines 16-0-1, 17-0-1, 17-0-5 and 18-2-4 were further evaluated under field conditions for their reactions to infection by PRSV and for fruit production. The data obtained from two round of field trials displayed that lines 16-0-1, 17-0-1, and 17-0-5 were highly resistant to PRSV. The total fruit yields in the second trials of each line harvested for six months showed a 3.0 to 3.2-fold increase, and the commercially valuable fruits a 54.3 to 56.7-fold increase (Yeh *et al.*, 1998). These results indicated that the CP transgenic papaya lines have a great potential for control of PRSV in Taiwan.

2.Control of PRSV by genetically-engineered mild strains

An attenuated strain, PRSV HA 5-1, that infects papaya without conspicuous symptoms was previously obtained by nitrous-acid induction from the severe parental Hawaii strain HA. It has been used successfully for control of PRSV by cross-protection on a large scale in Hawaii and Taiwan. However, strain-specific protection limits its application in Taiwan and other geographic areas (Yeh and Gonsalves, 1994; Yeh *et al.*, 1988). In order to analyze the genomic region responsible for the pathogenicity of PRSV, infectious *in vitro*

transcripts of both HA and HA5-1 were constructed and various virus hybrids between severe parental virus and its mild mutant were generated by recombination at the cDNA level. The genomic region responsible for the attenuation of HA5-1 was determined, analyzed and compared with parental severe strain HA. This genomic segment (nt606-3526) of HA 5-1 was transferred to and replaced the corresponding region of the infectious cDNA clone of Taiwan severe strain YK. The pathogenicity of the generated hybrid virus YKMF was demolished and induced symptoms similar to those caused by to HA5-1. The cross-protection effectiveness of YKMF was evaluated under green house condition. Over 80% of YKMF-protected papaya plants did not show symptom for 3 months after inoculation with different severe strains (Chen *et al.*, 2001). The result indicated that the attenuated strain YKMF provides much higher degree of cross-protection and is less strain-specific than HA5-1 and can potentially be used as a better protective strain than HA 5-1 to protect papaya from PRSV severe infection in Taiwan.

In another attempt to improve the cross protection effectiveness of HA 5-1 against heterologous strains, the CP gene of Taiwan type W-strain TW was amplified by RT-PCR and used to replace the CP gene of HA 5-1 at the cDNA level. The generated recombinant HA 5-1W also induced mild infections on papaya and cucurbits similar to that induced by HA 5-1. Under greenhouse conditions, results of cross protection tests indicated that HA 5-1W provides a better degree of protection against PRSV-W than HA 5-1 in *Citrullus lanatus* (Thunb.) Matsum & Nakai, and *Cucumis metuliferus* Naudin, respectively (You *et al.*, 2001). The results indicated that these recombinant mild strains could potentially be used to control the important PRSV-W strains in cucurbitaceous crops.

Conclusion

As the general public has become more concerned than before about environmental conservations, attention has been paid to the use of chemical pesticides especially the effect of their residue. Integrated pest management (IPM) has been regarded as an important approach to reduce the application of the agrochemicals, and non-pesticide control method is one of the principle components of the IPM. In recent years, development of non-pesticide measures has obtained some successful achievements those are now being adopted for agricultural production in various countries. To follow the global trend, our government has set the development and implementation of non-pesticide control an aim to safeguard our agriculture production and to protect our environment in the 21st century. As we have gained accession to the WTO in January, 2002, it is important for us to strengthen our competitiveness in agricultural production and international agricultural trade. The extensive application of IPM including non-pesticide-based crop protection methods will help reduce the consumption of chemical pesticides and produce safer

agricultural products appealing to consumers.

Being aware of the significance of non-pesticide control methods, Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ) has put great emphasis in supporting and coordinating research and extension projects in relation to this aspect since its establishment in 1998. Efforts have also been made to promote local awareness of non-pesticide control through demonstration and extension programs, and to educate farmers through training sessions and workshops. Some satisfactory research results were obtained and are ready for the application for patents for commercialization. To continue promoting non-pesticide control and bring it into practical application, some important issues still needs to be further addressed:

Firstly, how to integrate non-pesticide control methods into practical IPM models so that farmers are willing to adopt.

Secondly, how to formulate non-pesticide agents for commercial production so that they can be produced at low cost and applied without fast decline in efficacy over time.

Thirdly, What are the impacts to our ecosystem of the non-pesticide control methods especially that use introduced or genetically engineered agents.

Acknowledgment

The author thanks Dr. K. C. Lo and Dr. C. T. Lo at Taiwan Agricultural Research Institute, COA; Ms. C. C, Hung and Mr. M. L. Lee at Taiwan Agricultural Chemicals and Toxic Substances Research Institute, COA; Prof. J. W. Huang, Prof. Y. S. Lin, and Prof. S. D. Yeh at National Chun Hsing University for courteously providing their data to make this paper possible. The assistance of Ms. C. L. Chung, Mr. P. L. Chen, Dr. K. C. Kuo, and Ms. H. H. Lee at BAPHIQ in preparation of the paper is also appreciated.

Literature Cited

Chang, C. A. 2001. Application and extension of virus-free seedling for the control of virus diseases of passionfruit in Taiwan. *In* T.F. Hsieh, P. J. Ann, C. A. Chang & C. Y. Lin. eds. *Proceedings of the Symposium on Application of Healthy Plant for Control of Plant Diseases*. p. 31-38. Phytopathological Society of Republic of China. Taichung, Taiwan.

Chang, C. A., Chen, C. C., Yang, T. T., and Tsan, T. M. 2001. Production and application of virus free seeds to control virus diseases of asparagus beans. *In* T.F. Hsieh, P. J. Ann, C. A. Chang & C. Y. Lin. eds. *Proceedings of the Symposium on Application of Healthy Plant for Control of Plant Diseases*. p. 81-89. Phytopathological Society of Republic of China. Taichung, Taiwan.

Chen, C. C. 1992a. Ecology of *Trichogramma ostriniae* and its use for control of the Asian corn borer. *In Proceedings of Symposium on Non-pesticide*

Techniques for Control of Plant Diseases and Insect Pests. p. 5-14. Plant Protection Society of the Republic of China. Taichung, Taiwan.

Chen, W. S. & Chang, F. I. 1993. Control of melon fly (*Dacus cucurbitae* Coq.). *In* Liu, Y. C. ed. *Proceedings of Symposium on Vegetable Protection*. p. 169-181. Taichung, Taiwan.

Chen, Z. C. 1992b. Biological control of coconut leaf beetle by *Tetrastichus brontispae* Ferr. *In Proceedings of Symposium on Non-pesticide Techniques for Control of Plant Diseases and Insect Pests*. p. 31-41. Plant Protection Society of the Republic of China, Taichung, Taiwan.

Cheng, A. H., Wang, S. S. & Huang, S. N. 2001. The control of soil-borne diseases by grafting of tomato to eggplant rootstock. Tainan District Agricultural Services Bulletin 35: 1-3.

Cheng, A. H. & Yeh, C. C. 2001. Production and extension on bamboo mosaic virus-free clones/plants of green bamboo. *In* T.F. Hsieh, P. J. Ann, C. A. Chang & C. Y. Lin. eds. *Proceedings of the Symposium on Application of Healthy Plant for Control of Plant Diseases*. p. 75-80. Phytopathological Society of Republic of China. Taichung, Taiwan.

Chien C. C., Chiu S. C., & Ku S. C. 1989. Biological control of *Diaphorina citri* in Taiwan. Fruits. 44: 401-407.

COA.1996. *Non-pesticide Control of Crop Diseases and Insect Pests in Taiwan*. Taipei, Taiwan. 43 pp. Council of Agriculture. Taipei, Taiwan.

Ho, C. C. & Chen, W. H. 1992. Control of phytoseiids in a spider mite mass-rearing system (Acari: Phytoseiidae, Tetranychidae). Exp. Appl. Acarol. 13: 287-293.

Ho. C. C. & Lo, K. C. 1992. Biological control of spider mites in Taiwan. *In Proceedings of Symposium on Non-pesticide Techniques for Control of Plant Diseases and Insect Pests*. P. 15-29. Plant Protection Society of the Republic of China Taichung, Taiwan.

Hsiao, W. F. 1984. Application of *Bacillus thuringiensis* on cruciferous pest control. *In* R. F. Hsiao, C. M. Tien and S. J. Lin, eds. *Proceedings of the Symposium on the Insect Control of Vegetables in Taiwan*. p. 173-197. Bureau of Agriculture and Forestry, Taichung, Taiwan.

Huang, J. W. & Huang, H. C. 2000. A formulated container medium suppressive to *Rhizoctonia* damping-off of cabbage. Bot. Bull. Acad. Sinica 41:49-56.

Hung, C. C., & Hwang, J. S. 1993. Application of sex pheromone in pest management. *In* S. H. Huang, S. C. Hsieh & C. C. Chen, eds. *Proceedings of Symposium on Sustainable Agriculture*. p. 171-186. Taichung, Taiwan.

Huang, S. L., Cheng, A. H. & Chen, W. S. 1999. *Cultivation of Muskmelon in tunnel-shaped plastic structure*. Tainan District Agricultural Improvement Station Technical Bulletin No. 92. 28 pp.

Hwang, J. S. & Hung, C. C. 1992. Integrated control of sweet potato weevil, *Cylas formicarius*, with sex pheromone and insecticide. *In Proceedings of Symposium on Non-pesticide Techniques for Control of Plant Diseases and Insect Pests*. p. 81-94. Plant Protection Society of the Republic of China, Taichung, Taiwan.

Hwang, S. C. 2001. Application of tissue culture technology for controlling *Fusarium* wilt of banana. *In* T.F. Hsieh, P. J. Ann, C. A. Chang & C. Y. Lin. eds. *Proceedings of the Symposium on Application of Healthy Plant for Control of Plant Diseases*. p. 21-29. Phytopathological Society of Republic of China. Taichung, Taiwan.

Hwang, S. C. & Su, H. J. 1998. *Production of virus-free banana plantlets in Taiwan*. FFTC Extension Bulletin. No. 460, 7pp.

Kao, C. W. & Lee, H. H. 2001. The current status and perspectives of the system of healthy seeds and seedlings in Taiwan and the foreign countries. *In* T.F. Hsieh, P. J. Ann, C. A. Chang & C. Y. Lin. eds. *Proceedings of the Symposium on Application of Healthy Plant for Control of Plant Diseases*. p.1-11. Phytopathological Society of Republic of China. Taichung, Taiwan.

Lee, M. L. 1997. Studies on the control of soilborne fungal diseases by steam sterilization. *In Proceedings of the Symposium on Healthy Crops Cultivation*. p.112-127. Phytopathological Society of the Republic of China. Taichung, Taiwan.

Lin, Y. S., Huang, C. H & Soong, S. C. 1998. Resistance of bitter gourd-loofah grafts to *Fusarium oxysporum* f. sp. *momordicae* and their yield. Plant Prot. Bull. 40:121-132.

Lin, Y. S, Huang, C. S. & Kuo, M. S. 1996. Occurrence and dissemination of *Fuarium* wilt of bitter gourd in Taiwan. Plant Path. Bull. 5: 38-46.

Liu, S. D. 1991. Biological control of adzuki-bean root rot disease caused by *Rhizoctonia solani*. Plant Prot. Bull. 33: 63-71.

Liu, Y. C. 1991. Current status and control of the oriental fruit fly, Dacus dorsalis Hendel, of Taiwan. *In Proceedings of International Symposium on Biological Control of Fruit Fly*. p. 81-86. Okinawa, Japan.

Lo, K. C.. 2002. Biological control of insect and mite pests on crops in Taiwan. *In* W. T. Wu ed. *Proceedings of the Symposium on the Biological Control of Agricultural Insects and Mites*. p. 1-25. Taiwan Entomological Society. Taipei, Taiwan.

Lo, K. C., Lee, W. T., Wu, T. K., & Ho, C. C. 1990. Use of predators to control spider mites (Acarina: Tetranychidae) in the Republic of China on Taiwan. P.166-178 *In* O. Mochida, K. Kiritani & J. Bay-Petersen eds. *The Use of Natural Enemies to Control Agricultural Pests*. FFTC Book Series. No. 40.

Lo, C. T. 2001. Development and application of Trichoderma spp. for plant disease control in Taiwan. *In* D. S. S. Tzeng & J. W. Huang. eds. *Proceedings of International Symposium on Biological Control of Plant Diseases for the New Century-Mode of Action and Application Technology*. p. 85-96. National Chung University. Taichung, Taiwan.

Su, H. J. 2001. Production and extension system of virus-free citrus seedling. *In* T.F. Hsieh, P. J. Ann, C. A. Chang & C. Y. Lin. eds. *Proceedings of the Symposium on Application of Healthy Plant for Control of Plant Diseases*. p. 13-19. Phytopathological Society of Republic of China. Taichung, Taiwan.

Sun, S. K. & Huang, J. W. 1983. Effects of soil amendments on *Fusarium* wilt of watermelon. Plant Prot. Bull. 25:127-137.

Sun, S. K. & Huang, J. W. 1985. Formulated soil amendment for controlling *Fusarium* wilt and other soilborne diseases. Plant Dis. 69:917-920.

TARI. 1999. *Biological Control: Introduction of Research and Application of Natural Enemies*. Taiwan Agri. Res. Inst. Spec. Pub. No. 80. 63 pp. Taiwan Agricultural Research Institute. Taichung, Taiwan.

Tsay, T. T. 1997. Sustainable utilities in control plant pathogenic nematodes with organic amendments. *In Proceedings of the Symposium on Healthy Crops Cultivation*. p. 154-162. Phytopathological Society of the Republic of China. Taichung, Taiwan.

Tsay, T. T. 1998. Biological Control of Plant Parasitic Nematodes. National Taiwan University, Taipei, Taiwan. (Ms thesis). 101 pp.

Tzeng, D. D. S., Huang, J. W. & Tzeng, K. C. 2001. Development of antagonistic bacteria as biofungicide for the control of plant diseases. *In* D. S. S. Tzeng & J. W. Huang. eds. *Proceedings of International Symposium on Biological Control of Plant Diseases for the New Century-Mode of Action and Application Technology*. p. 107-126. National Chung University. Taichung, Taiwan.

Wang, C. L. & Lin, F. C. 1992. The application of yellow sticky cards on the management of *Liriomyza* flies. *In Proceedings of Symposium on Non-pesticide Techniques for Control of Plant Diseases and Insect Pests*. .p. 99-103. Plant Protection Society of the Republic of China, Taichung, Taiwan.

Wang, D. N. & Wang, H. L. 1995. Control of papaya ringspot virus by using screenhouse cultivation in Taiwan. *In Proceedings of International Symposium on Integrated Management of Insect-borne Virus Diseases of Tropical Fruits*. p.26 (Abstract). Taichung, Taiwan.

Wang, H. L., Yeh, S. D. & Chiu, R. J. 1992. Cross protection techniques for control of plant virus diseases in Taiwan. *In Proceedings of Symposium on Non-pesticide Techniques for Control of Plant Diseases and Insect Pests*. .p. 317-324. Plant Protection Society of the Republic of China, Taichung, Taiwan.

Wang, H. L., Yeh, S. D., Chiu, R. J. & Gonsalves, D. 1987. Effectiveness of cross protection by mild mutants of papaya ringspot virus for control of ringspot disease of papaya in Taiwan. Plant Dis. 71:491-497.

Wu, Y. R. & Tseng, C. T. 1997. The application of *Bacillus thurigiensis* mixed with fried rice bran on the control of Asian corn borer. Tainan District Agricultural Services Bulletin 21: 18-22.

Yeh, S. D., Bau, H. J., Cheng, Y. H., Yu, T. A. & Yang, J. S. 1998. Greenhouse and field evalutions of coat-protein transgenic papaya resistant to papaya ringspot virus. Acta Horticulturae 461:321-328.

Yeh, S. D. & Gonsalves, D. 1994. Practices and perspective of control of papaya ringspot virus by cross protection. Adv. Dis. Vector Res. 10:237-257.

Yeh, S. D., Gonsalves, D., Wang, H. L., Namba, R. & Chiu, R. J. 1988. Control of papaya ringspot virus by cross protection. Plant Dis. 72:375-380.

You, B. J., Chian, C. H., Su, W. C., Chen, L. F., and Yeh, S. D. 2001.

Enhancement of the degree of cross protection against type W strain of *Papaya ringspot virus* by the chimeric mild strain of type P virus carrying heterologous 3'-terminal untranslation region from type W strain. Plant Pathology Bulletin: 10: 211.

Table 1. The records of introduced natural enemies during 1971-1995 in Taiwan

Year	Natural enemy	Target of pests	Origin	Status [a]
1971	*Opius importatus*	Beanflies	Hawaii	F
1971	*Opius phaseli*	Beanflies	Hawaii	F
1973	*Apanteles vestalis*	Diamondback moth	Holland	F
1973	*Diadegma fenestralis*	Diamondback moth	Holland	F
1974	*Apanteles glomeratus*	Imported cabbage worm	Japan	E
1983	*Tetrastichus brontispae*	Coconut leaf beetle	Guam	S
1983	*Tamarixia radiata*	Citrus psylla	Reunion	S
1983	*Trissolcus basalis*	Green stingbug	Hawaii	E
1983	*Amblyseius californicus*	Two-spotted spider mite	Hawaii	E
1983	*Typhlodromus occidentalis*	Two-spotted spider mite	US	F
1984	*Trichogramma nubilale*	Asian corn borer	US	F
1985	*Amblyseius fallacis*	Two-spotted spider mite	US	S
1985	*Diadegma eucerophaga*	Diamondback moth	Indonesia	E
1987	*Cotesia erionotae*	Banana skipper	Hawaii	E
1987	*Ooencyrtus erionotae*	Banana skipper	Hawaii	E
1988	*Dacnusa sibirica*	American leafminer	Holland	F
1988	*Encarsia formosa*	Greenhouse whitefly	Holland	F
1988	*Trichogrammatoidea bactrae fumata*	Litchi fruit borer	Thailand	E
1988	*Curinus coeruleus*	Jumping plantlice	Hawaii	F
1989	*Typhlodromus occidentalis*	Two-spotted spider mite	US	-
1989	*Phytoseiulus persimilis*	Two-spotted spider mite	US	S
1990	*Phytoseiulus persimilis*	Two-spotted spider mite	Australia	S
1990	*Nephaspis annicola*	Spiraling whitefly	Hawaii	F
1990	*Nephaspis bicolor*	Spiraling whitefly	Hawaii	F
1993	*Macrocentrus grandii*	Asian corn borer	US	F
1995	*Encarsia haitiensis*	Spiraling whitefly	Hawaii	-
1995	*Encarsia guadelopupae*	Spiraling whitefly	Hawaii	-

[a] S stands for successful in control, F stands for failure in establishment, E stands for established, and – stands for status uncertain.

Table 2. The application of sex pheromones and attractants in Taiwan

Sex pheromones or attractants	Purposes	Extension (ha) [a]
Tobacco cutworm (*Spodoptera litura* Fabricius)	Monitoring Mass trapping: 6-8 traps / ha / month	22,000
Beet army worm (*Spodoptera exigua* Hubner)	Monitoring Mass trapping: 6-24 traps / ha / 1-2months	1,700
Tomato fruitworm (*Helicoverpa armigera* Hubner)	Monitoring Mass trapping: 6-24 traps / ha / 1-2months	350
Sweet potato weevil [*Cylas formicarius* (Fabricius)]	Monitoring Mass trapping : 40 traps / ha / 1-2months	2,800
Carambola fruit borer (*Eucosma notanthes* Meyrick)	Monitoring Mass trapping: 40~50 traps / ha / 5~8months Mating disruption: 120 spots/ 0.1 ha / 5 months	400
Smaller tea tortrix (*Adoxophyes* sp.)	Monitoring Mass trapping: 25~40 traps / ha / month	250
Tea tortrix (*Homona magnanima* Diakonoff)	Monitoring Mass trapping: 25~40 traps / ha / month	1,350
Asian corn borer (*Ostrinia furnacalis* Guenée)	Monitoring	—
Oriental fruit moth (*Grapholita molesta* Busck)	Monitoring	—
Litchi fruit moth (*Cryptophlebia ombrodelta* (Lower))	Monitoring	—
Methyl Eugenol (95%) and Naled (5%)mixture	Monitoring: 549 detection points Trapping and killing; 4~6 pic/ha/2 months	580,000
Cuelure (85%)	Monitoring: 162 detection points Trapping and killing: 4~6 pic/ha/2 months	Commonly used
Methyl Eugenol 95%, Cuelure 85% (1:1) and Naled 5% mixture	Monitoring: 36 detection points Mass trapping and killing: 4 pic/ha/2 months	1,550

[a] The numbers are the total acreages that the methods had been applied to under the subsidiary of the government from 1998 to 2001.